Biological Consulting Services
of North Florida, Inc.

May 5, 2009

Aphex BioCleans Systems, Inc.

Dear Sirs,

We have completed the antiviral efficacy study on the supplied DermAphex hand Sanitizer. The testing was done according to the protocol we briefly discussed and have used previously in disinfectant studies. The protocol used is comparable to ASTM E 1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Surfaces). Bacteriophage PRD-1 was used as a surrogate to Influenza A virus to determine efficacy of disinfection. According to the observed results the supplied DermAphex Hand Sanitizer exhibited significant antiviral properties. In the following pages, you will find a summary of the methodology used and the results of our analysis.

Should you have any further concerns please do not hesitate to contact me.

Best Regards,

George Lukasik, Ph.D.
Laboratory Director

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Stock Virus and Cell Culture Preparation

Bacteriophage PRD-1 virus was propagated and enumerated as plaque forming units (pfu) using EPA 1601described methodology. Salmonella typhimurium (ATCC 19585) was used as the host. Viruses were enumerated using a double layer plaque assay. Virus enumeration and propagation was done by incubation on the appropriate host at 36.5°C for 24hr. Plaques were counted following incubation and the pfu/ml was calculated. At the day of challenge experiment virus stocks (typically 1x 10^9 pfu/ml) are diluted 1/1000 in Class 1 ASTM reagent water supplemented with 1% fetal bovine serum (FBS, Atlanta Biologicals, GA).

Challenge Study; May 05, 2009

The protocol used is comparable to ASTM E 1053-97 (Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Surfaces). Briefly, two hundred and fifty microliters of the above virus dilution was evenly spread on the surface of 50 mm plastic Petri dishes. The Petri dishes were then allowed to incubate for 10 minutes at 25° C. Following, 500 µl of Dermaphex was spread evenly onto the inoculated plates using a cell scraper/spreader. The plates were then allowed to incubate for a total contact time of 3 minutes while being agitated. Four milliliters of Neutralizing Buffer (Beckton Dickinson, MD) was added to each plate at the end of the 3 minute contact

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time. The liquid on each plate was agitated by repeated pipetting. The liquid was removed from each plate and placed in a sterile 50 ml centrifuge tube (Fisher scientific, PA) containing 15 ml sterile Neutralizing Buffer. Ten fold dilutions of the viral suspensions were performed in PBS. The number of viable PRD-1 virus was in each of the tubes was enumerated by plaque assay procedure. All analysis was conducted in triplicates. Plates containing viral inoculums and no DermAphex treatment were used as negative controls. The recovered viable viral pfu from the negative control plates were used to calculate challenge concentration and percent reduction. Table 1 below presents the results of the above-mentioned test.

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Table 1. The efficacy of Dermaphex hand sanitizer during a 3 minute contact time on the inactivation of PRD-1 bacteriophage.

Treatment	PRD-1 Average pfu/ml*
Untreated (Negative Control)	2.0×10^4
Dermaphex	4.3×10^0
Percent Reduction	99.98 %

*Data represents an average of three trials for each test point. Plaque forming units (pfu) of PRD-1 were enumerated using EPA 1601comparable Methodology using S. typhimuriun as a host. PRD-1 Bactriophage was used a s a surrogate for Influenza virus inactivation.

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